Comprehensive Phytochemical and Antioxidant Profiling of *Picrorhiza kurroa*

Deepanshi Pathak[1] and Rachana R[1]*
[1, 1*]Jaypee Institute of Information Technology, Noida, India, deepanshipathak14@gmail.com
*Corresponding Author: rachana.dr@iitbombay.org

Abstract:

Several medicinal plants have multiple bioactive compounds that offer potential therapeutic applications for various respiratory-associated pathologies. *Picrorhiza kurroa* is one of those important traditional medicinal herbs and is known for its potent bioactive phytoconstituents against respiratory and other inflammatory disorders. Current study was aimed to perform phytochemical profiling and antioxidant potential of various organic and aqueous extracts of *P. kurroa* roots. Qualitative studies confirmed the presence of various phytochemical groups: triterpenoids, alkaloids, flavonoids, phenols, and carbohydrates. Quantitative evaluation showed that the ethanolic and methanolic extracts exhibited higher phytochemical content in comparison to aqueous extracts. Chromatographic profiling using TLC identified that ethanolic extract contained apocynin while it was absent in methanolic and aqueous extracts. While evaluating antioxidant potential, ethanolic extract was found to have the highest potential in comparison to the methanolic extract. The result suggests that the ethanolic extract of *P. kurroa* is a rich source of antioxidants and active phytoconstituent apocynin in comparison to other extracts and can be a preferred extract to be used to neutralize reactive oxygen species.

Keywords: Phytochemicals, Qualitative tests, Quantitative tests, Antioxidant potential, Respiratory disorders, Inflammation, *P. kurroa,* Organic extracts, Aqueous extract.

Introduction

The essential function of the respiratory system is to supply adequate oxygen to bodily tissues, for normal organ functions and oxygenation. Due to its large gas-exchange surface, the respiratory system is particularly prone to oxidative stress-induced damage. High pulmonary oxygen levels and inhaled exogenous pollutants promote the formation of reactive oxygen species (ROS) and reactive nitrogen species (RNS) (Moran *et al*., 2025). In a healthy physiological state, a certain level of ROS plays a vital role in maintaining redox homeostasis. An internal balance of reactive species and antioxidants actively shields cells from the damaging effects of oxidative stress. ROS are overproduced due to excessive stimulation of mitochondrial electron transport chain dysfunction and xanthine oxidase activity or reduced nicotinamide adenine dinucleotide phosphate by pro-inflammatory cytokines such as interleukin-1β and tumor necrosis factor-α. Both endogenous and exogenous pro-oxidants induce cellular activation, leading to the release of pro-inflammatory mediators and proteases. It also activates host defense mechanisms, utilizing enzymatic and non-enzymatic antioxidant systems (Taniguchi *et al.,* 2021). These processes interact within a multilevel cycle that regulates oxidant-antioxidant homeostasis. Disruption of this balance results in oxidative stress, a deleterious condition that induces airway and lung tissue damage and contributes to the

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pathogenesis of several inflammatory respiratory diseases, including asthma, chronic obstructive pulmonary disease (COPD), cystic fibrosis and acute respiratory distress syndrome (ARDS) (Lee *et al.*, 2010).

Clinically used anti-inflammatory drugs, such as corticosteroids (like methylprednisolone), have adverse effects (neuromuscular weakness, gastrointestinal bleeding) and expensive treatment (Kuperminc *et al.,* 2023). There is a long history of using medicinal herbs to treat respiratory conditions. In the modern era of medicine, herbal remedies play a significant role in enhancing life expectancy by improving the immune system. Remarkably, over 50% of all clinically approved drugs are derived from natural products, highlighting their indispensable role in modern drug discovery and development within the pharmaceutical industry (Gohel *et al.,* 2021).

Picrorhiza kurroa, traditionally known as kutki, has been used in Ayurveda since ancient times for managing respiratory ailments such as allergies and liver-related disorders (Debnath *et al.,* 2020). It is valued in traditional medicine for its wide range of pharmacological properties, including antioxidant, anti-inflammatory, antibacterial, antitumor, antidiabetic and hepatoprotective effects (Singh *et al.,* 2022). The most economically important parts for this herb are its rhizomes and dried roots, which possess a bitter taste and are widely used for managing liver disorders, respiratory allergies, upper respiratory tract infections, and spleenrelated conditions (Debnath *et al.,* 2020). The major phytochemical research has been caried out on rhizomes but the roots of *P. kurroa* are also a rich source of bioactive compounds. Roots are often the primary site for the synthesis of phenolic and tannins to protect the plants from soil-borne pathogens. Also, acknowledging the medicinal value of the roots ensures that no part of this endangered resource should be wasted. Phytochemical studies have revealed that this plant contains several bioactive compounds, including picroside I and II, apocynin, Dmannitol, cucurbitacin glycosides, kutkiol, and kutki sterol. Among these, apocynin, is a major source present in the roots of *P. kurroa* and is a catechol derivative, recognized as a potent antiinflammatory molecule. This compound has the ability to prevent the production of ROS molecules (Yildrim et al., 2025). It has been shown to mitigate ischemia-reperfusion injury, hypoxia, and Parkinson's disease by inhibiting NADH oxidase activity triggered under oxidative stress (Almeleebia *et al.,* 2022).

In the current study, various extracts including aqueous and organic were prepared and further both qualitative and quantitative phytochemical evaluations were conducted to detect and measure the bioactive constituents present. Chromatographic profiling was carried out using TLC, HPTLC and HPLC. The antioxidant potential of these extracts was assessed through multiple *in vitro* assays, including DPPH, ABTS, FRAP and hydrogen peroxide (H_2O_2) scavenging methods.

2. Materials and Methods

2.1. Collection of plant material and its extraction

The dried root powder was purchased from Natural Remedies Private Limited (Bangalore, Karnataka, India). Root powder of *P. kurroa* (16 grams) was subjected to extraction with

various organic solvents (250ml) (Chloroform, Ethanol, Methanol, Ethyl acetate, and Petroleum ether) for 7 days in a rotary shaker at room temperature and distilled water (DW) for 6 hours in a water bath at 37°C (Kant et al. (2013)).

The extraction procedure for organic solvents (maceration) is of 7 days because it depends upon passive diffusion. While aqueous extraction (heat) is fast as it is carried out in hot water, which increases both the kinetic energy and the diffusion rate. Organic solvents have low polarity and kinetic energy, that is why it takes longer for the solvent to penetrate the plant material and disuse out of the compounds.

2.2. Preliminary Phytochemical Screening

A qualitative study was conducted on the following extracts: petroleum ether, ethanol, methanol, chloroform, ethyl acetate, and aqueous, in order to discover different active ingredients, such as tannins, alkaloids, steroids, flavonoids, phenols, and glycosides using the methods mentioned in the table below (table1).

Table 1. Preliminary phytochemical screening tests

Phytochemical group	Test name	Procedure	Positive indication	Reference
Carbohydrates	Molisch's test	Plant extract + alcoholic α-naphthol + conc. H_2S0_4	Violet ring at layer junction	(Veerachari and Bopaiah , 2011)
	Benedict's test	Plant extract + Benedict's reagent, boiled in water bath	Reddish brown precipitate	(Veerachari and Bopaiah, 2011)
	Fehling's test	Plant extract + Fehling's A & B, boiled	Brick red precipitate	(Ramarajan *et al.,* 2019)
Proteins	Millon's test	Plant extract + Millon's reagent, heated gently	White ppt. turning crimson	(Karthikeyan and Vidya *et al.,* 2019)
Alkaloids	Wagner's Test	Plant extract + Wagner's reagent	Reddish brown precipitate	(Karthikeyan and Vidya *et al.,* 2019)
Phenolic compounds	Zinc-HCl Reduction	Plant extract + zinc dust + conc. HCl	Yellow/ orange coloration	(Ramya *et al.,* 2019)
	Ferric Chloride test	Plant extract + 5% $FeCl_3$ solution	Bluish black/ dark green color	(Ramya *et al.,* 2019)
Anthocyanins	Heat test	Plant extract + 10% NaOH, heated at 100°C for 5 min	Blue coloration	(Redha *et al.,* 2018)
Sterols & Triterpenoids	Salkowski reaction	Plant extract + chloroform + conc. H_2SO_4	Red lower layer = sterols Yellow layer = triterpenoids	(Rahimah *et al.,* 2019)

Tannins	Ferric Chloride test	Plant extract + 5% $FeCl_3$ solution	Blue (gallic), greenish black (catecholic), brownish green (condensed tannins).	(Ramya *et al.,* 2019)
	Alkaline Reagent test	Plant extract + alkaline $FeCl_3$ solution	Green blue (catecholic), blue (gallic), green brown (condensed)	(Ramya *et al.,* 2019)
Quinones	Sulphuric acid test	Plant extract + conc. H_2S0_4	Red coloration	(Roghini and Vijayalakshmi *et al.,* 2019)
Flavonoids	Zinc-HCl reduction	Plant extract + zinc dust + conc. HCl	Red coloration	(Pant *et al.,* 2017)
	Alkaline Reagent test	Plant extract + 10% NaOH/ NH_4OH, then dilute acid	Yellow to colorless	(Pant *et al.,* 2017)

2.3. Quantitative Analysis

Quantitative analysis of the aqueous, ethanol, and methanol extracts of *P. kurroa* was performed to identify the optimal solvent for the highest yield of TPC, TFC and TTC as per the methods mentioned in table 2.

Table 2. Quantitative phytochemical screening

Quantitative Analysis	**Method**	**Procedure**	**Absorbance**	**Reference**
Total Phenolic	Folin-Ciocalteu method	Plant extract + Folin-Ciocalteu reagent + 7%	720 nm	(Madhu *et al.,* 2016)
Content (TPC)		Na_2CO_3 solution (incubate for 2 hours)		
Total Flavonoid Content (TFC)	Aluminium chloride colorimetric method	Plant extract + 5% $NaNO_3$ (incubate for 5 minutes) + 10% $AlCl_3$ + 1M NaOH	510 nm	(Madhu *et al.,* 2016)
Total Tannin Content (TTC)	Folin-Ciocalteu method	Plant extracts + Folin-Ciocalteu + 35% Na_2CO_3 (incubate for 5 minutes)	700 nm	(Cl and Indira, 2016)

2.4. Thin Layer Chromatography (TLC) and High-Performance Thin Layer Chromatography (HPTLC)

2.4.1 TLC analysis was performed on silica gel G (stationary phase) plates using the mobile phase [Toluene: Ethyl acetate: Methanol: Formic acid (40: 50: 10: 0.2)]. After development to three-fourths of the plate, spots were visualized under UV light at 254 nm (Mallick et al., 2015). Dragendroff's reagent revealed a prominent green spot corresponding to apocynin, and the retention factor (R_f) was calculated using:

$$R_f = \text{(distance travelled by the compound)}/\text{(distance travelled by the solvent)}$$

2.4.2 HPTLC analysis was further performed to confirm the results of TLC. HPTLC analysis was performed on silica gel G (stationary phase) plates using the mobile phase same as used in TLC. The scanning was performed at 254 nm using a CAMAG TLC scanner 3 equipped with WinCats software.

2.5. High Performance Liquid Chromatography (HPLC)

HPLC analysis for the quantification of vasicine was performed using a Hewlett-Packard 1100 Series HPLC System (Hewlett-Packard, CA, USA). Sample injections were carried out using a Rheodyne HP7725 manual injector fitted with a 20 µl loop. Separation was achieved on a C18 column (4.6 x 250mm; 15 µm pore size). The mobile phase consisted of acetonitrile and water with acetic acid (60:40, v/v) at a flow rate of 1 ml/min. The column was maintained at ambient temperature, and detection was performed at 276 nm using a photodiode array (Oliveira et al., 2017).

2.6. Antioxidant Activity

2.6.1. Determination of DPPH radical scavenging activity

The antioxidant activity was assessed using the DPPH radical scavenging method, as described by Blois (1958), with slight modifications. Plant extracts and ascorbic acid (20-100 µg/ml) were mixed with 0.1 mM DPPH solution and incubated in the dark at room temperature for 30 minutes. Absorbance was recorded at 517 nm, and radical scavenging activity (%) was calculated using:

$$\% \text{ inhibition} = [(A_0 - A_1 -)/A_0] \times 100$$

where, A_0 is the absorbance of the control, and A_1 is the absorbance sample

The inhibitory concentration (IC_{50}) DPPH values (the concentration of the sample required for 50% inhibition of the DPPH radical) were obtained from the linear regression line. This IC_{50} value was used to assess the antioxidant activity.

2.6.2. Estimation of H_2O_2 radical scavenging activity

The experiment was conducted in accordance with Ruch et al. (1989) instructions. Absorbance at 230nm was determined after plant extracts (20-100 µg/ml) were combined with phosphate buffer (pH 7.4) and 2mM H_2O_2 solution, and then incubated for 10 minutes. Scavenging activity (%) was computed using the following formula with ascorbic acid serving as the standard.

$$\text{Scavenging percentage} =[(A_0-A_1–)/A_0] \times 100$$

2.6.3. Determination of ABTS

The experiment was carried out with a few minor adjustments in accordance with Ohikhena (Ohikhena *et al.,* 2018). After 7mM ABTS and 2,45 Mm $K_2S_2O_8$ (1:1, v/v) reacted, the mixture was incubated in the dark for 12-16 hours to produce ABTS+. At 734 nm, the solution's absorbance was 0.700±0.020 after being diluted with a 1:1 ethanol-water. ABTS solution (1:1, v/v) was combined with extracts and ascorbic acid (standard) (20-100 µg/ml), incubated for 6 minutes, and absorbance was measured at 734 nm. Percentage inhibition was calculated using:

$$(\%) \text{ inhibition} = [(A_0 - A_1)/A_0] \times 100$$

2.6.4. Ferric Reducing Antioxidant Power (FRAP) assay

The FRAP assay was conducted using, with minor adjustments, the Oyaizu method. In short, 0.2 ml of extract at several concentrations (20-100 µg/ml) was combined with 0.5 ml of 1% potassium ferricyanide and 0.5 ml of phosphate buffer (0.2 M, pH 6.6). The mixture was incubated at 50°C in a water bath for 20 minutes, then cooled to room temperature.
Subsequently, 250 µl of 10% trichloroacetic acid was added, and the mixture was centrifuged at 3000 rpm for 10 minutes. From the supernatant, 250 µl was collected, mixed with 250 µl of distilled water, and combined with 50 µl of 0.1 % ferric chloride. After incubation at room temperature for 10 minutes, the absorbance was recorded at 700 nm. Ascorbic acid was used as the standard reference.

3. Results and Discussion

3.1. Phytochemical Screening

Phytochemical screening is crucial for identifying and assessing the constituents in plant extracts, especially bioactive compounds with potential therapeutic applications. In this study, qualitative analysis confirmed the presence of various bioactive molecules in *P. kurroa* root extracts across different solvents (Table 3). Carbohydrates, phenols, triterpenoids, proteins, tannins, quinones, and flavonoids were found in ethanolic, methanolic, and aqueous extracts. Chloroform and petroleum ether extracts contained similar compounds, including carbohydrates, sterols, triterpenoids, quinones, and flavonoids. The ethyl acetate extract showed quinones and flavonoids. Overall, aqueous, ethanol, and methanol extracts exhibited a wider range of phytoconstituents compared to chloroform, petroleum ether, and ethyl acetate extracts.

The therapeutic effects of *P. kurroa* are likely linked to these bioactive molecules. Terpenoids are known for various pharmacological activities such as anti-inflammatory,

antibacterial, anticancer, and cholesterol-lowering effects. Flavonoids have notable antioxidant properties and can inhibit tumor initiation, promotion, and progression, while tannins display antiviral, antibacterial, and antitumor activities. Rathee *et al.,* in 2016, revealed the presence of flavonoids, carbohydrates, phenols, proteins, and tannins in the methanolic extract of *P. kurroa* rhizomes. Various researchers, such as Agarwal *et al.* (2023), Sharma *et al.* (2018), Deb *et al.* (2018), and Thakur *et al.* (2018) also showed the presence of these phytochemicals in the *P. kurroa.*

Based on these findings, the ethanolic, methanolic, and aqueous extracts were chosen for further quantitative phytochemical evaluation due to their higher content of bioactive compounds. The observed differences in phytochemical composition among solvents underscore the variable solubility and extractability of these compounds, which may, in turn, affect their pharmacological potential.

Table 3: Qualitative analysis of *P. kurroa* extracts

Phytochemicals		Ethanolic	Methanolic	Chloroform	Petroleum ether	Ethyl acetate	Aqueous
1. Carbohydrates	Molisch test	+	+	+	+	-	+
	Benedict test	+	+	-	-	-	+
	Fehling test	+	+	-	-	-	+
2. Proteins	Millon's test	+	+	-	-	-	+
3. Alkaloids	Wagner's test	-	-	-	-	-	-
4. Phenolic compounds	Zinchydrochloride reduction test	-	-	-	-	-	-
	Ferric chloride test	+	+	-	-	-	+
5. Anthocyanins	Heat test	-	-	-	-	-	-
6. Sterols	Salkowski reaction	-	-	-	+	-	-
7. Triterpenoids	Salkowski reaction	+	+	+	-	-	+
8. Tannins	Ferric chloride test	+	+	-	-	-	+
	Alkaline reagent test	-	-	-	-	-	-
9. Quinones	Sulphuric acid test	+	+	+	+	+	+
10. Flavonoids	Zinchydrochloride reduction test	-	-	-	-	-	-

	Alkaline reagent test	+	+	+	+	+	+

+ Present; - Absent

3.2. Quantitative Analysis

Among the six tested extracts, the methanolic, ethanolic, and aqueous extracts displayed the greatest diversity of phytoconstituents and were therefore selected for quantitative evaluation. The quantitative phytochemical profile of *P. kurroa* extract confirms the presence of major secondary metabolites in moderate quantities. Phenols, flavonoids and tannins were quantified and are presented in Table 4.

The ethanolic extract showed the highest levels of phenolics (26.09±0.25 mg GAE/g), followed by the methanolic extract (21.21±0.21 mg GAE/g). The calibration curve for the standard is shown in Fig 1. Phenols exhibit antioxidant activity by playing a critical role in the adsorption and neutralization of free radicals. These compounds also demonstrate significant antibacterial, antidiabetic and anticholinergic activities (Nisar *et al.,* 2022). TFC was observed to be highest in the ethanolic extract (81.76±0.22 mg QE/g), whereas lowest in the aqueous extract (62.71±0.18 mg QE/g).

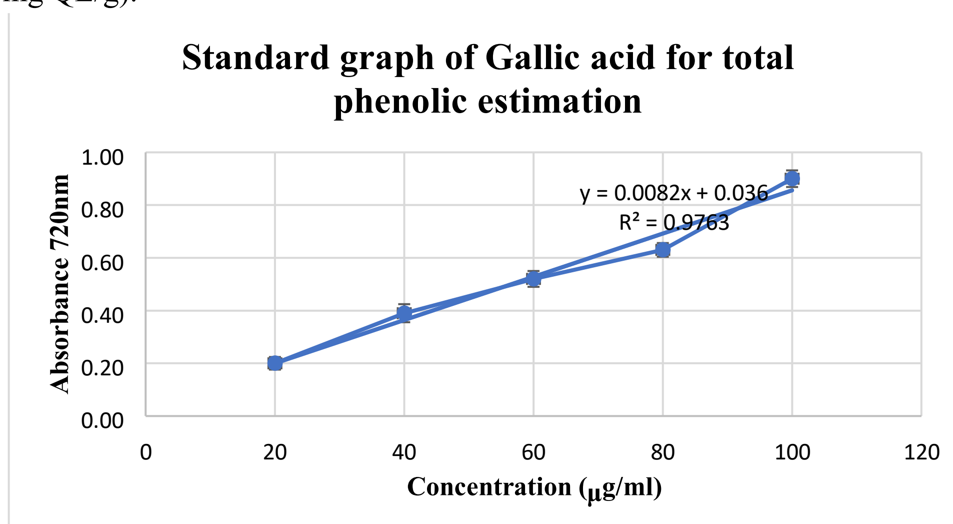

Fig 1. Calibration curve for the standard Gallic acid for TPC

The analysis of total flavonoid content showed that the ethanolic extract (81.76±0.22 mg QE/g) had a significantly higher total flavonoid content followed by methanolic extract (67.47±0.19 mg QE/g). Flavonoids are well known for their biological activities such as antioxidant, antibacterial, hepatoprotective, anticancer, anti-inflammatory and antiviral properties (Shrestha et al., 2024). In a similar study, Krupashree *et al.,* (2014) quantified the TPC and TFC in the ethanolic extract of *P. Kurroa.* Rajkumar *et al.,* (2011) identified significant total phenolic content in the methanolic extract of *P. kurroa*. Neupane and Lamichhane (2020) also observed the presence of both TPC and TFC in the methanolic extract of the plant. Nisar *et al.,* (2022), reported that the methanolic leaf extract of *P. kurroa* possesses high concentrations of both TPC and TFC.

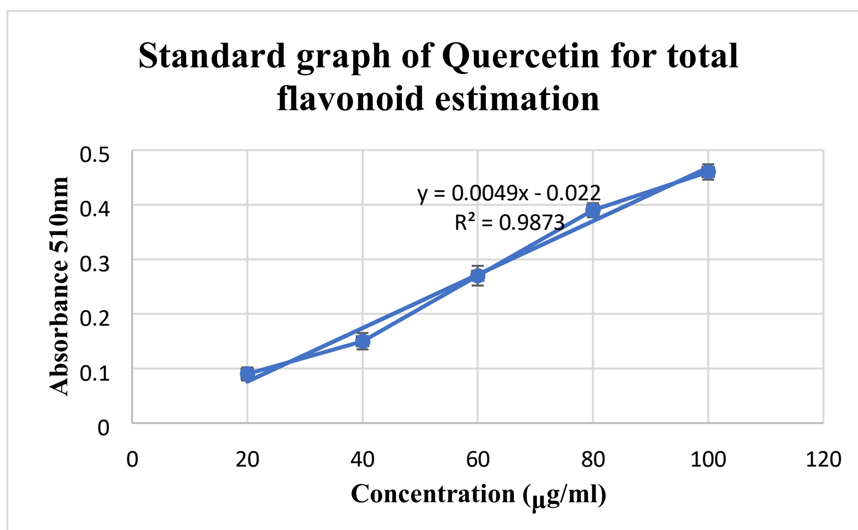

Fig 2. Calibration curve for the standard quercetin for TFC

Maximum tannins were found in the ethanolic extract of *P. kurroa* (56.41±0.2mg GAE/g), followed by the methanolic (51.28±0.18 mg GAE/g) and aqueous extract (41.02±0.14 mg GAE/g). The calibration curve for the standard gallic acid is shown in Fig 3. Tannins, another important group of phenolics, exhibit strong antibacterial and antiviral effects and possess notable free radical scavenging activity, contributing to cellular protection and antiageing functions (Sun *et al.,* 2023). In a study of *P. kurroa* rhizomes, Pokhriyal *et al.,* (2023) evaluated the total tannin content in the ethanolic extracts obtained from the three different collection sites.

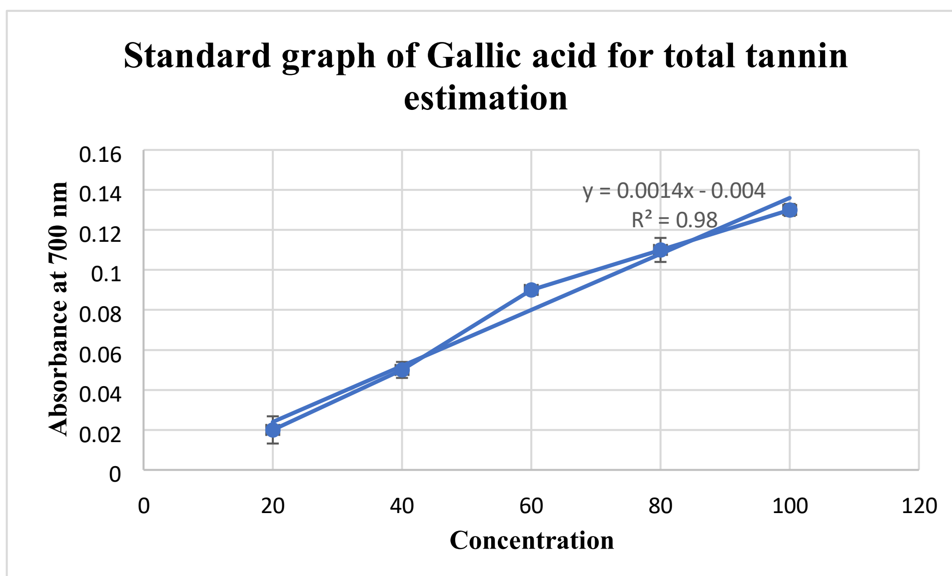

Fig 3. Calibration curve for the standard Gallic acid for TFC Table 4. Total phenol, flavonoid and tannin content

Extracts	TPC mg of Gallic acid equivalents/g dry weight of plant extract	TFC mg of Quercitin equivalents/g dry weight of plant extract	TTC mg of Gallic acid equivalents/g dry weight of plant extract

P. kurroa ethanolic extract	26.09±0.25	81.76±0.22	56.41±0.20
P. kurroa methanolic extract	21.21±0.21	67.47±0.19	51.28±0.18
P. kurroa aqueous extract	11.46±0.13	62.71±0.18	41.02±0.14

3.3. TLC and HPTLC

The presence of apocynin in *P. kurroa's* aqueous, ethanolic and methanolic extracts was analysed using TLC. The solvent system effectively resolved the bioactive compounds present in the extracts. The identification of apocynin in the extracts was confirmed by comparing the retention factor (R_f) value with that of the apocynin standard. The R_f value of Apocynin was found to be 0.72, whereas in the ethanolic extract R_f value was observed to be 0.70, which matched the R_f value of the pure compound (apocynin). Nevertheless, the methanolic and aqueous extracts showed no bands, indicating that apocynin was not present in this solvent system. Mallick *et al.* (2015) observed well-defined peaks of apocynin with an R_f value of 0.77.

Based on these results, HPTLC was carried out for the ethanolic extract to validate and evaluate the presence of apocynin in the extract. Figure 4 depicts the results of HPTLC, where the dark band shows the presence of apocynin, whereas the light band indicates the presence of apocynin in the ethanolic extract of *P. kurroa*. The variation in band shade reflects the higher concentration in the pure compound compared to the extract.

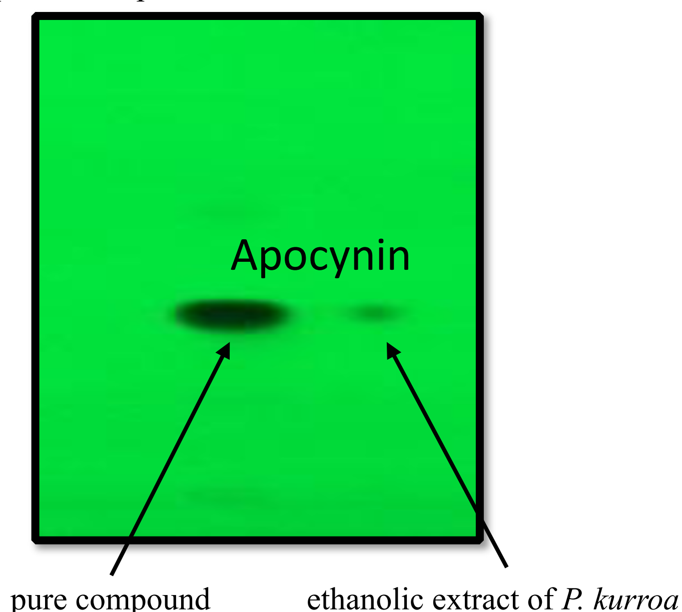

Figure 4: HPTLC chromatogram of pure compound apocynin and apocynin in ethanolic extract of *P. kurroa*

3.6. HPLC

Apocynin is a key bioactive marker in the roots of *P. kurroa*, which was quantified using a developed HPLC technique. The analysis utilizes a mobile phase consisting of acetonitrile and

water with acetic acid with a ratio of 60:40. The retention time (RT) for the marker compound apocynin was found to be 4.59 minutes. The HPLC chromatogram of the ethanolic extract exhibited a RT-4.59 minutes, which is the same as that of the standard marker as shown in Figure 5. Quantification of apocynin through HPLC analysis showed its content to be 0.029% in the ethanolic extract of *P. kurroa.*

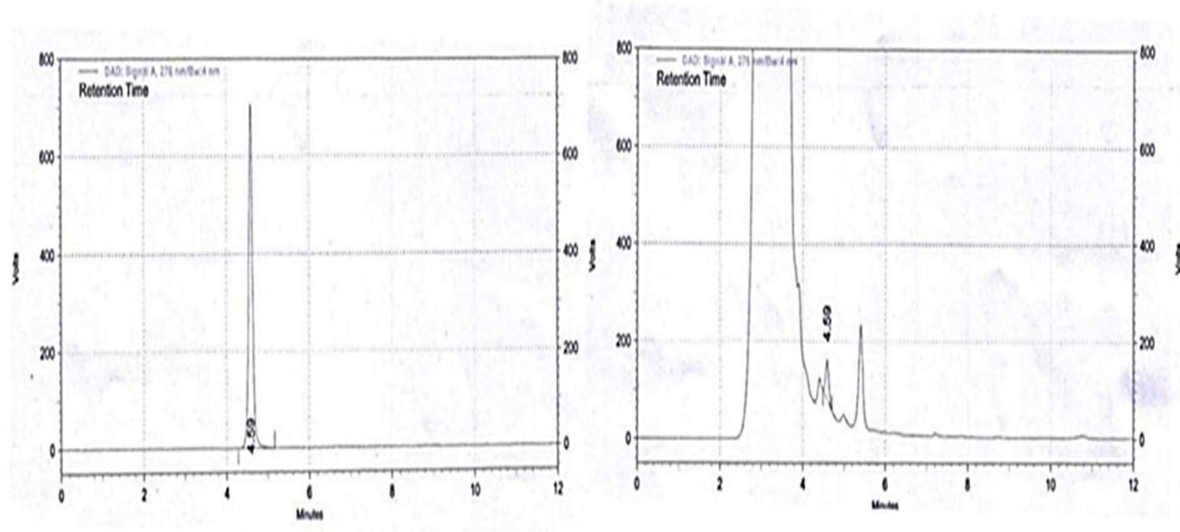

Figure 5: HPLC chromatogram (a) ethanolic extract of *P. kurroa* (b) apocynin

The system suitability was verified through the comparative analysis of the apocynin standard and ethanolic extract of *P. kurroa* extract. Both the chromatograms show the target analyte eluting at a consistent retention time of 4.59 minutes. Visual inspection of the peaks confirms high symmetry (tailing factor ≈1.0) and resolution is more than 2.0. These parameters indicate that the HPLC system and the theoretical plates were optimal for the precise quantification of apocynin.

3.3. Antioxidant Activity

Based on the quantitative results, ethanolic and methanolic extracts were further analysed for antioxidant activity using DPPH, ABTS, H_2O_2 and FRAP. Their ability to scavenge the free radicals at different concentrations was analyzed. The percentage inhibition of absorbance was calculated and plotted as a function of concentration of the extract and of standard. Table 5 shows the results of IC_{50} values of DPPH, ABTS and H_2O_2. The concentration of antioxidants required to trap 50% of DPPH, ABTS and H_2O_2 absorbance is known as the IC_{50}, and it was used to express the antioxidant capacity. In this context, a lower IC_{50} value reflects higher antioxidant efficiency. The performance of each extract was evaluated and compared against ascorbic acid, which served as the standard.

Ethanolic extracts showed lower IC_{50} values in comparison to methanolic extracts in DPPH, ABTS and H_2O_2 antioxidant assays performed, which indicates that the ethanolic extract exhibits stronger antioxidant activity than the methanolic extract.

The DPPH assay measures the radical scavenging ability of antioxidants by monitoring the decrease in DPPH absorbance, accompanied by a colour change from purple to yellow, which reflects the conversion of DPPH to its stable, reduced form via hydrogen donation (Bajpai et

al., 2015). The IC_{50} values of ascorbic acid, ethanolic extract and methanolic extract determined by DPPH assay were 26.57 ± 0.95, 63.59 ± 1.14 and 73.33 ± 0.98 µg/ml respectively (Figure 6). The results demonstrated that the methanolic and ethanolic extracts showed less antioxidant activity as compared to the standard. The DPPH radical, a stable free radical, is widely used as a quick and sensitive assay to assess the free radical scavenging potential of both lipophilic and hydrophilic antioxidants. Antioxidants can neutralize DPPH radicals either by electron transfer or by donating hydrogen atoms (Banerjee et al., 2005).

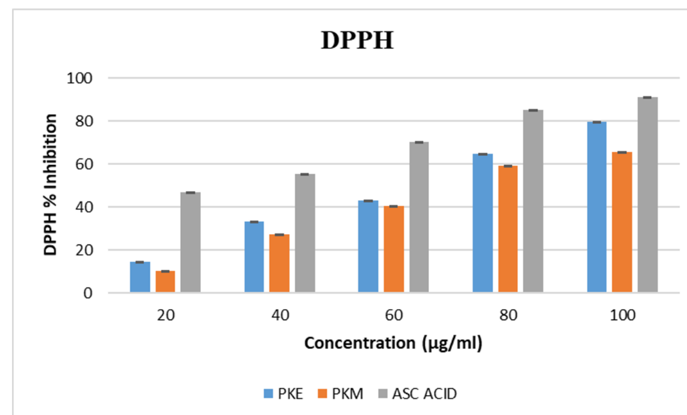

Figure 6: DPPH Assay
Comparative scavenging of DPPH radical by ascorbic acid, ethanolic extract of *P. kurroa* (PKE), and methanolic extract of *P. kurroa* (PKM)

The IC_{50} values of ascorbic acid, ethanolic extract and methanolic extract determined by ABTS assay were 15.03 ± 0.99, 40.08 ± 1.42 and 67.53 ± 1.11 µg/ml respectively (Figure 7). Ethanolic extract showed more antioxidant activity in comparison to methanolic extract and less than standard. The ABTS + radical scavenging capacity of the extracts was assessed in comparison with the standard antioxidant Trolox. The ABTS radical cation was generated using potassium persulfate and stabilized until a constant absorbance was reached. The plant extracts were then added, and the antioxidant activity was determined by measuring the degree of decolourization (Rajurkar and Hande, 2011).

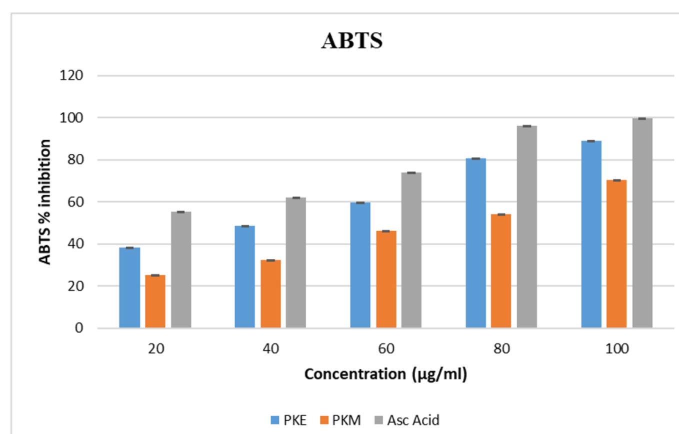

Figure 7: ABTS Assay
Comparative scavenging of ABTS radical by ascorbic acid, ethanolic extract of *P. kurroa* (PKE), and methanolic extract of *P. kurroa* (PKM)

The IC_{50} values of the standard ascorbic acid, ethanolic extract and methanolic extract determined by H_2O_2 assay were 53.55 ± 0.91, 90.5 ± 1.1 and 109.7 ± 1.24 µg/ml respectively (Figure 8). Methanolic extract showed less antioxidant than ethanolic extract. Hydrogen peroxide (H_2O_2) is a mild oxidizing agent capable of inactivating certain enzymes by oxidizing essential thiol (-SH) groups. Its small size allows it to cross cell membranes rapidly, where it can interact with intracellular Fe^{2+} and Cu^{2+} ions, generating highly reactive hydroxyl radicals responsible for much of its toxicity. The scavenging of H_2O_2 by plant extracts is attributed to bioactive secondary metabolites, particularly phenolics, which donate electrons to neutralize H_2O_2, converting it into water (Ruskin R, 2017).

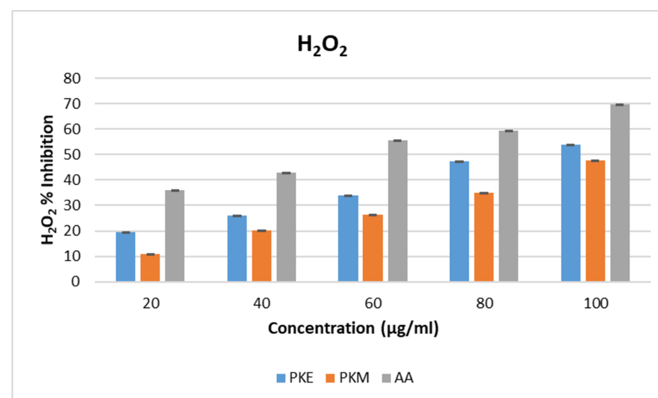

Figure 8: H_2O_2 Assay
Comparative scavenging of H_2O_2 radical by ascorbic acid, ethanolic extract of *P. kurroa* (PKE), and methanolic extract of *P. kurroa* (PKM)

The FRAP antioxidant activity of *P. kurroa* ethanolic and methanolic extract was evaluated by their ability to reduce Fe^{3+} to Fe^{2+}, observed as a color change from yellow to green or blue colour, with intensity increasing alongside extract concentration. Results (Figure 9) showed that both the extract and standard antioxidant compounds enhanced reducing power with concentration, with the ethanolic extract exhibiting a stronger antioxidant effect compared to the methanolic extract. The ferric reducing antioxidant power (FRAP) assay evaluates the reducing potential of antioxidants by measuring their ability to convert the ferric tripyridyl triazine complex (Fe^{3+} -TPTZ) to its ferrous form (Fe^{2+} - TPTZ), resulting in a blue coloured complex. This reaction occurs at acidic pH (approx. 3.6) and reflects the antioxidants hydrogen atom donation capacity, with maximum absorbance observed at 593 nm (Rajurkar and Hande, 2011).

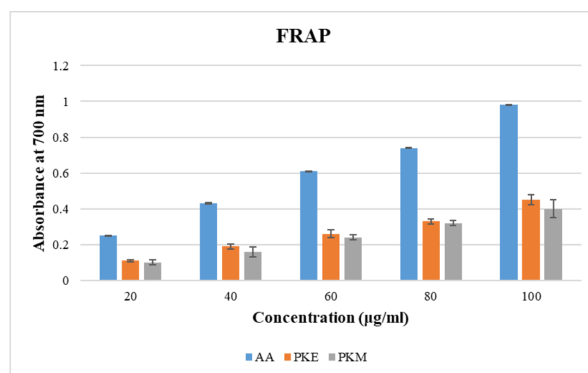

Figure 9: FRAP Assay
Comparative scavenging of FRAP radical by ascorbic acid, ethanolic extract of *P. kurroa* (PKE), and methanolic extract of *P. kurroa* (PKM)

Kant *et al.* (2013) observed promising results in the leaves of *P. kurroa* in terms of DPPH and ABTS. Krupashree *et al.* (2014) quantified the DPPH radical scavenging (IC_{50} = 75.16 ± 3.2 µg/ml) and FRAP in the ethanolic root extract of *P. kurroa*. antioxidants Rajkumar *et al.* (2011) also observed antioxidant potentials (DPPH and FRAP) in the methanolic and aqueous extracts of rhizomes of *P. kurroa*. Summary of these antioxidant assays is shown in table 5.

Table 5: IC_{50} values of ethanolic and methanolic extract of *P. kurroa* along with the standard ascorbic acid

Extracts	DPPH (IC_{50} µg/ml)	ABTS (IC_{50} µg/ml)	H_2O_2 (IC_{50} µg/ml)
P. kurroa ethanolic extract	63.59 ± 1.14	40.08 ± 1.42	90.5 ± 1.1
P. kurroa methanolic extract	73.33 ± 0.98	67.53 ± 1.11	109.7 ± 1.24
Ascorbic acid	26.57 ± 0.95	15.03 ± 0.99	53.55 ± 0.91

To establish a mechanistic link between the secondary metabolite profile and biological efficacy, a Pearson correlation analysis was performed. A very strong positive correlation was observed between TPC and TTC (Pearson coefficient = 1.0), indicating a coextraction of these polyphenolic fractions. Furthermore, a strong inverse correlation (Pearson coefficient= -1.0) was found between phytochemical density (TPC/TFC/TTC) and the IC_{50} values of DPPH, ABTS and H_2O_2 assays. This statistically validates that the superior antioxidant capacity of the ethanolic extract is a direct consequence of its enriched polyphenolic and flavonoid content, supporting the use of these compounds a bioactive marker for *P. kurroa.*

Conclusion

This study demonstrates that the ethanolic extract of *P. kurroa* possesses the most promising fraction of phytochemical profile in comparison to other extracts. Qualitative and quantitative analyses confirmed a high abundance of bioactive secondary metabolites, which directly

correlated with the significant antioxidant activity observed across DPPH, ABTS, H_2O_2 and FRAP assays. By chromatographic profiling, the quantity of apocynin was confirmed, which possesses anti-oxidant activity. These findings, achieved through standard laboratory techniques, validate the therapeutic potential of *P. kurroa* as a cost-effective and accessible candidate for the treatment of respiratory diseases. This highlights the urgent need for further research to clarify apocynin and ethanolic extract of *P. kurroa's* pharmacodynamics and safety in human pathologies.

Conflict of Interest

There is no conflict of interest among the authors.

References

Agrawal S, Rana PS and Saklani P (2023). Exploration of Leaves of *Picrorhiza kurroa* for their Medicinal Potential by Phytochemical Analysis and Antibacterial Activity. *Env. Eco.,* 41 (4B): 2693-2700.

Almeleebia TM, Alsayari A and Wahab S (2022). Pharmacological and Clinical Efficacy of *Picrorhiza kurroa* and Its Secondary Metabolites: A Comprehensive Review. *Mol.*, 27(23): 8316.

Bajpai VK, Agrawal P, Bang BH and Park YH (2015). Phytochemical analysis, antioxidant and antilipid peroxidation effects of a medicinal plant, *Adhatoda vasica*. *Front. Life Sci.*, 8(3): 305–312.

Banerjee A, Dasgupta N, De B (2005). In vitro Study of Antioxidant Activity of *Syzygium cumini* fruit. *Food Chem*., 90(4): 727-733.

Cl KC and Indira G (2016). Quantitative estimation of total phenolic, flavonoids, tannin and chlorophyll content of leaves of *Strobilanthes Kunthiana* (Neelakurinji). *J. Med. Plants Stud.*, 4(4): 282–286.

Deb K, Kaur A, Ambwani S and Ambwani TK (2018). Preliminary phytochemical analyses of hydro-methanolic leaf extract of *Melia azedarach* L. *J. Med. Pl. Stud.,* 6: 4-8.

Debnath P, Rathore S, Walia S, Kumar M, Devi R and Kumar R (2020). *Picrorhiza kurroa*: a promising traditional therapeutic herb from higher altitude of western Himalayas. *J. Herb. Med.*, 23: 100358.

Gohel KH, Patel PB, Shah PM, Patel JR, Pandit N and Raut A (2021). Knowledge and perceptions about COVID-19 among the medical and allied health science students in India: An online cross-sectional survey. *Clin. Epidemiol. Glob. Health.*, 9: 104–109.

Lee T, Luo SF, Lee CW *et al* (2010). Overexpression of HO-1 Protects against TNF-αMediated Airway Inflammation by Down-Regulation of TNFR1-Dependent Oxidative Stress. *Am. J. Pathol.,* 175(2): 519-532.

Kant K, Walia M, Agnihotri VK, Pathania V and Singh B (2013). Evaluation of antioxidant activity of *Picrorhiza kurroa* (leaves) extracts. *Indian J. Pharm. Sci.,* 75(3): 324-329.

Karthikeyan G and Vidya AK (2019). Phytochemical Analysis, Antioxidant and Antibacterial activity of Pomegranate peel. *Res. J. Life Sci. Bioinform.*, 05(01): 218–231.

Krupashree K, Kumar KH, Rachitha P, Jayashree GV, Khanum F (2014) Chemical composition, antioxidant and macromolecule damage protective effects of *Picrorhiza kurroa* Royle-ex Benth. *S. Afr. J. Bot.,* 94: 249–254.

Kuperminc E, Heming N, Carlos M and Annane D (2023). Corticosteroids in ARDS. *J. Clin. Med.*, 12(9): 3340.

Madhu M, Satyadev TNVSS, Satyanarayana MV (2016). Quantitative phytochemical analysis of selected medicinal plant species by using various organic solvents. *J. Pharmacogn. Phytochem.*, 5(2): 25–29.

Mallick MN, Singh M, Parveen R, Khan W, Ahmad S, Zeeshan Najm M and Husain SA (2015). HPTLC Analysis of Bioactivity Guided Anticancer Enriched Fraction of Hydroalcoholic Extract of *Picrorhiza kurroa*. *Biomed Res Int*., 2015(1): 513875.

Moran MO, Perez E, Arranz AE and Garrido A (2025). The Role of Reactive Oxygen Species in Lung Cancer Development: Nanomedicine as a Therapeutic Strategy. *Biomolecules,* 15(9): 1316.

Nisar J, Shah SMA, Akram M, Ayaz S and Rashid A (2022). Phytochemical screening, Antioxidant, and Inhibition Activity of *Picrorhiza kurroa* against α-Amylase and αGlucosidase. *Dose-Response,* 20(2): 15593258221095960.

Ohikhena FU, Wintola OA and Afolayan AJ (2018). Quantitative Phytochemical Constituents and Antioxidant Activities of the Mistletoe, Phragmanthera capitata (Sprengel) Balle Extracted with Different Solvents. *Pharmacogn. Res.*, 10(1): 16–23.

de Oliveira JK, Ronik DFV, Ascari J, Mainardes RM and Khalil NM (2017). A stabilityindicating high performance liquid chromatography method to determine apocynin in nanoparticles. *J. Pharm. Analysis*, *7*(2), 129-133.

Pant D, Pant N, Saru D, Yadav U and Khanal D (2017). Phytochemical screening and study of anti-oxidant, anti-microbial, anti-diabetic, anti-inflammatory and analgesic activities of extracts from stem wood of Pterocarpus marsupium Roxburgh. *J. Intercult. Ethnopharmacol.*, 6(2): 1.

Pokhriyal A, Prakash S and Patni B (2023). Comparative Study on the Biochemical Profile and Antioxidant Activity of *Picrorhiza kurrooa* Rolye ex Benth. Obtained from Uttarakhand. *Evid. Based Complement Alternat Med.,* 1(2023): 8792414.

Rajurkar NS and Hande SM (2011). Estimation of Phytochemical Content and Antioxidant Activity of Some Selected Traditional Indian Medicinal Plants. *Indian J. Pharm. Sci.,* 73: 146151.

Rahimah SB, Djunaedi DD, Soeroto AY and Bisri T (2019). The Phytochemical Screening, Total Phenolic Contents and Antioxidant Activities in Vitro of White Oyster Mushroom (Pleurotus Ostreatus) Preparations. *Open Access Maced. J. Med. Sci.*, 7(15): 2404–2412.

Rajkumar V, Guha G and Kumar RA (2011). Antioxidant and anti-neoplastic activities of *Picrorhiza kurroa* extracts. *Food Chem. Toxicol.,* 49(2): 363-369.

Ramarajan S, Janakiraman V and Allan (2019). Quantitative Phytochemical Analysis of different Solvent extracts of *Turbinaria ornata* -A Marine Macroalgae. *Res. J. Pharm. Tech.*, 12(1): 108.

Ramya P, Vasanth PM, Prasad PV and Babu SV (2019). Qualitative Phytochemical Screening tests of *Alpina Galanga* L. *World J. Pharm. Res.*, 8(5): 1064–1077.

Redha AAA, Hasan AM, and Mandeel Q (2018). Phytochemical Determinations of Pomegranate (*Punica granatum*) Rind and Aril Extracts and their Antioxidant, Antidiabetic and Antibacterial Activity. *Nat. Prod. Chem. Res.*, 06(04).

Roghini R and Vijayalakshmi K (2018). Phytochemical Screening, Quantitaive Analysis of Flavonoids and Minerals in Ethanolic Extract of *Citrus paradisi*. *Int. J. Pharm. Sci. Res.*, 9(11): 4859–4864.

Sharma R, Deb K, Ambwani TK and Ambwani S (2018) Preliminary phytochemical screening and antioxidative potential of *Picrorhiza kurroa* Royale ex. Benth, *Bull Environ. Pharmacol. Life Sci.,* 7:134–139.

Shrestha D, Magar AB, Pakka S and Sharma KR (2024). Phytochemical analysis, antioxidant, antimicrobial, and toxicity studies of *Schima wallichii* growing in Nepal. *Int. J. Food Prop.*, 27(1): 273–285.

Singh G, Bisht D, Arya RKK, Kumar S, Kumar D and Dash AK (2022). Identification and Quantification of six natural compounds from *Picrorhiza kurroa* leaf extract and their Antibacterial and Antioxidant activity. *Res. J. Pharm. Tech.*, 5774–5778.

Sun Y, Feng JX, Wei ZB, Sun H, Li L, Zhu JY, Xia GQ and Zang H (2023). Phytochemical Analysis, Antioxidant Activities In Vitro and In Vivo, and Theoretical Calculation of Different Extracts of *Euphorbia fischeriana*. *Mol.*, 28(13): 5172.

Taniguchi A, Tsuge M, Miyahara N and Tsukahara H (2021). Reactive Ixygen Species and Antioxidant Defense in Chronic Obstructive Pulmonary Disease. *Antioxidants*, 10(10): 1537.

Thakur AV, Ambwani S and Ambwani TK (2018). Preliminary phyto-chemical screening and GC-MS analysis of leaf extract of *Acacia catechu* (Lf.) *Willd. Int. J. Herb. Med.,* 6: 81–85.

Yildrim BA, Dogan T, Bolat I, Ozcan AC and Kocak R (2025). Effect of NADPH Oxidase Inhibitor Apocynin on Human Lung Cancer A549 Cells via Bcl-2, Bax, Caspase-3, and NF-B Signaling Pathway. *Naunyn Schmiedebergs Arch. Pharmacol.,* 398: 10071-10082.

Veerachari U and Bopaiah AK (2011). Preliminary phyto-chemical evaluation of the leaf extract of five Cassia Species. *J. Chem. Pharm. Res.*, 3(5): 574–583.

Vasanthkumari B (2017). In vitro Antioxidant Activity of Various Leaf Extracts of Canthium coromandelicum (Burm. F.) Alston. *Asian J. Pharm. Clin. Res.,* 10(5): 214-218.